2021

Role of food web interactions in promoting resilience to nutrient enrichment in a brackish water eelgrass (Zostera marina) ecosystem

K. Gagnon

C. Gustafsson

(...)

J. P. Richardson

P. L. Reynolds

See next page for additional authors

Authors
K. Gagnon, C. Gustafsson, (...), J. P. Richardson, P. L. Reynolds, J. E. Duffy, and C. Boström

LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 66, 2021, 2810–2826

doi: 10.1002/lno.11792

Role of food web interactions in promoting resilience to nutrient enrichment in a brackish water eelgrass (*Zostera marina*) ecosystem

Karine Gagnon,[1*] **Camilla Gustafsson**,[2] **Tiina Salo**,[1] **Francesca Rossi**,[3] **Sonja Gunell**,[1] **J. Paul Richardson**,[4] **Pamela L. Reynolds**,[4,5] **J. Emmett Duffy**,[4,6] **Christoffer Boström**[1]

[1]Environmental and Marine Biology, Åbo Akademi University, Åbo, Finland
[2]Tvärminne Zoological Station, University of Helsinki, Hanko, Finland
[3]ECOSEAS Laboratory, CNRS, Université de la Côte d'Azur, Nice, France
[4]Virginia Institute of Marine Science, College of William & Mary, Virginia
[5]DataLab: Data Science and Informatics, University of California, Davis, California
[6]Tennenbaum Marine Observatories Network, Smithsonian Environmental Research Center, Edgewater, Maryland

Abstract

Understanding the ecological interactions that enhance the resilience of threatened ecosystems is essential in assuring their conservation and restoration. Top-down trophic interactions can increase resilience to bottom-up nutrient enrichment, however, as many seagrass ecosystems are threatened by both eutrophication and trophic modifications, understanding how these processes interact is important. Using a combination of approaches, we explored how bottom-up and top-down processes, acting individually or in conjunction, can affect eelgrass meadows and associated communities in the northern Baltic Sea. Field surveys along with fish diet and stable isotope analyses revealed that the eelgrass trophic network included two main top predatory fish species, each of which feeds on a separate group of invertebrate mesograzers (crustaceans or gastropods). Mesograzer abundance in the study area was high, and capable of mitigating the effects of increased algal biomass that resulted from experimental nutrient enrichment in the field. When crustacean mesograzers were experimentally excluded, gastropod mesograzers were able to compensate and limit the effects of nutrient enrichment on eelgrass biomass and growth. Our results suggest that top-down processes (i.e., suppression of algae by different mesograzer groups) may ensure eelgrass resilience to nutrient enrichment in the northern Baltic Sea, and the existence of multiple trophic pathways can provide additional resilience in the face of trophic modifications. However, the future resilience of these meadows is likely threatened by additional local stressors and global environmental change. Understanding the trophic links and interactions that ensure resilience is essential for managing and conserving these important ecosystems and the services they provide.

Both resource availability (bottom-up) and predation pressure (top-down) are fundamental ecological processes determining community structure and biodiversity (Hairston et al. 1960; Hillebrand et al. 2007). It is well established that human activities increase nutrient loading (through eutrophication) and food web alterations (through the overexploitation of top predatory species), which can significantly modify the strength and relative importance of bottom-up and top-down forcing in coastal vegetated ecosystems (Hauxwell et al. 1998; Heck et al. 2000; Reynolds et al. 2014). However, a remaining major challenge is to understand to what extent their individual or combined roles shape community structure and trophic networks in different key habitats. Seagrass ecosystems have emerged as a model system for testing the relative importance of bottom-up and top-down forcing in marine environments (e.g., Whalen et al. 2013; Duffy et al. 2015; Yan et al. 2020).

Seagrass meadows provide multiple important ecosystem services critical for human well-being, but are being lost at accelerating rates due to human activities (Nordlund et al. 2016; Unsworth et al. 2019). Although the mechanisms behind seagrass loss vary between ecosystems and regions (Orth et al. 2006; Waycott et al. 2009), there is substantial evidence that changes in bottom-up control through nutrient pollution can shift seagrass ecosystems to an algal-dominated state (see review by Burkholder et al. 2007) with subsequent

*Correspondence: karine.gagnon@abo.fi

Additional Supporting Information may be found in the online version of this article.

losses in ecosystems goods and services (Rönnbäck et al. 2007; Schmidt et al. 2017). Specific loss mechanisms involve reductions in water clarity, along with increased epiphytic loading and shading, caused by micro- and macro algal blooms, that reduce seagrass growth and increase mortality (Thomsen et al. 2012; Gustafsson and Boström 2014; Bittick et al. 2018).

In pristine seagrass meadows, Yan et al. (2020) showed that intact populations of top predators confer resilience by limiting the negative effects of experimental bottom-up nutrient enrichment (Yan et al. 2020). However, in many seagrass meadows, the overexploitation of top predators can disrupt these trophic interactions that enhance resilience to eutrophication (Moksnes et al. 2008; Baden et al. 2012). Specifically, there is mounting evidence that the overfishing of top predatory fish leads to seagrass loss by increasing intermediate predators abundance, which in turn reduce the abundance of invertebrate mesograzers in the community. These mesograzers are critical for promoting seagrass resilience to eutrophication by consuming epiphytic algae, thus increasing light availability and enhancing seagrass production (Moksnes et al. 2008; Baden et al. 2012; Östman et al. 2016), as well as higher biodiversity through increased habitat provisioning (Valentine and Duffy 2006; Reynolds et al. 2014). Mesograzer losses due to trophic cascades, thus strongly impact seagrass productivity and associated biodiversity negatively and strengthen the negative impacts of nutrient enrichment.

Despite evidence from correlative studies supporting a negative impact of nutrient addition on seagrass (Hughes et al. 2004), many nutrient addition experiments have failed to demonstrate significant effects in the field despite substantial increases in local nutrient concentrations (e.g., Williams and Ruckelshaus 1993, Heck et al. 2000; Baden et al. 2010), suggesting the importance of context-dependency. Nutrient dilution effects due to hydrodynamic settings, experimental design, and/or rapid grazing of epiphytes which transfer nutrients into mesograzers biomass, may all contribute to the difficulty in detecting bottom-up forcing (Baden et al. 2010; Reynolds et al. 2014; Duffy et al. 2015). Thus, concurrent experimental manipulations of bottom-up and top-down processes is critical for a mechanistic understanding of the effects of anthropogenic drivers on a given seagrass ecosystem.

The coasts of the Nordic countries support at least 1500–2100 km^2 of eelgrass *Zostera marina* (Boström et al. 2014), but extensive declines in eelgrass coverage and depth limits have been recorded throughout the North Sea and southern Baltic Sea (de los Santos et al. 2019). In Denmark, the present distribution constitutes approximately only 10–20% of the historical distribution, while 60% has been lost along the Swedish Skagerrak coast, due to seagrass wasting disease in the first half of the 20th century (Muehlstein 1989), and overfishing and eutrophication in the second half. More recently, climate change including marine heat waves, poses new challenges to seagrass meadows (Smale et al. 2019). The pathogen that causes wasting disease is not present in the brackish-water northern Baltic Sea (Jakobsson-Thor et al. 2018, 2019), but despite high levels of eutrophication and trophic cascades, there are no apparent large-scale declines in this area (Boström et al. 2014). Interestingly, this regional pattern of eelgrass decline (high losses in the Skagerrak and Kattegat, relatively stable populations in the northern Baltic) strikingly matches the regional scale variation in food web alterations (overfishing of top predators and increases in intermediate predators) and the geographic distribution of eelgrass mesograzer biomass, which are higher and lower, respectively, in the Skagerrak/Kattegat than northern Baltic (Jephson et al. 2008; Baden et al. 2010; Boström et al. 2014). These observations further support the idea that the effects of bottom-up nutrient enrichment are strongly mediated by top-down processes, but the exact mechanisms are still unknown.

Here, we investigated how changes in bottom-up (nutrients) and top-down (grazing) processes affect temperate eelgrass ecosystems in the northern Baltic Sea, using a combination of manipulative field experiments complemented by fish and invertebrate surveys and stable isotope analysis. Empirical work on the structure of seagrass food webs has been widespread (e.g., Vizzini et al. 2002; Hoshika et al. 2006; Vafeiadou et al. 2013), but only a few studies have provided mechanistic insights by combining stable isotope analysis with experimental manipulations of nutrients and/or predation pressure (e.g., Armitage and Fourqurean 2009, Howard et al. 2016), and none in the Baltic region. We explored these topics to address the following specific research questions:

1. What is the trophic structure of the northern Baltic eelgrass food web and what are the main energy flow pathways between primary producers, grazers, and higher-level consumers?
2. How do nutrient addition and grazer exclusion (i.e., changes in bottom-up and top-down control, respectively), acting individually and in conjunction, influence food web interactions, biodiversity, eelgrass biomass and growth, and biomass of epiphytic and drift filamentous algae?

Assuming that mesograzers are resource-limited, we expected their abundance to be highest in the nutrient addition treatment and lowest in the mesograzer exclusion treatment. As mesograzers feed on and control the abundance of filamentous algae and epiphytes, we thus expected eelgrass biomass and growth to be highest in the control treatment without manipulation and lowest in the treatment with both nutrient addition and grazer exclusion. Conversely, we expected the abundance of filamentous drift and epiphytic algae to be lowest in the control treatment and highest in the nutrient addition + grazer exclusion treatment. Further, we expected that the negative effects of nutrient addition and grazer exclusion on eelgrass would also have negative effects on associated invertebrate biodiversity.

Materials and methods

Study area and experiment sites

The study area is located in the Finnish Archipelago Sea, northern Baltic Sea, a geographically complex region

characterized by over 25,000 islands and skerries. Eelgrass grows mainly on exposed sandy bottoms at 2–5 m depth (Boström et al. 2006) in the middle and outer archipelago zones (Granö et al. 1999), where the annual surface water temperature and salinity range between 0–24°C and 6–7, respectively. This study was conducted over two eelgrass growing seasons (2011 and 2012), at two sites (Table 1): Ängsö (60.1091 N, 21.7109 E) and Fårö (59.9197 N, 21.796242 E) with large (> 5 ha) meadows at 2–5 m depth, dominated by eelgrass growing intermixed with *Ruppia cirrhosa*, *Stuckenia pectinata*, *Potamogeton perfoliatus*, and *Zannichellia palustris* (Boström et al. 2006). Both sites are semi-exposed and the sediment is sand with low silt and organic content (Table 1). Porewater nutrients have been measured in several studies with NH_4^+ values ranging from 20–60 μM (Gustafsson and Boström 2011, 2014).

For detailed regional and site descriptions, see previous study in this region (e.g., Boström et al. 2006, Gustafsson and Boström 2011, 2014). A larger detailed study was first conducted at Ängsö in 2011 to (a) quantify the structure of the food web, and (b) mechanistically study the effects of nutrient addition and mesograzer exclusion. We then repeated the field experiment at a second site (Fårö) in 2012 to assess the generality of the results. Not all response variables were assessed during the second year (see below).

Invertebrate and fish surveys

Invertebrates

We quantified the ambient invertebrate community in the eelgrass meadow at Ängsö in late June and early August 2011, using a 20-cm diameter mesh bag (mesh size = 1 mm) that was placed over the eelgrass shoots, and cutting shoots at the sediment surface. These samples (n = 10) target epifaunal invertebrates, but also incidentally sample infaunal species (especially bivalves) that are on or near the sediment surface. We sorted and separated the eelgrass from the invertebrates, measured eelgrass dry weight, then identified and counted all macroinvertebrates (> 1 mm) at the lowest taxonomic level possible (species or genus, except insect larvae which were identified to family). Invertebrate densities were then standardized to individuals g dw^{-1} *Z. marina*. We similarly sampled and quantified the invertebrate community from the control plots (n = 10 per site) in the field experiment (see below), in September 2011 (Ängsö) and 2012 (Fårö).

Fish and stomach contents

At Ängsö in 2011, we used several different sampling methods to quantify the fish community present in the eelgrass meadow. To identify the main fish predators we used multimesh Nordic gill nets (1.8 m high, 45 m long). These nets consist of a series of 5 m long, randomly distributed mesh panels (nine panels with mesh sizes of 10, 12, 15, 19, 24, 30, 38, 40, and 60 mm). The nets were deployed within the eelgrass meadow at 2–4 m depth, parallel to the shoreline. To study the temporal dynamics of the fish community, we used diurnal gill net sampling (six sampling cycles within 24 h: 15:00–19:00, 19:00–23:00, 23:00–03:00, 03:00–07:00, 07:00–11:00, 11:00–15:00) once a month in June, July, and August. We also deployed gill nets within the meadow before (early August) and after (early September) the field experiment during a 12 h cycle (overnight). In addition, to catch smaller (< 5 cm) intermediate predators, we complemented the gill net surveys with beach seining (seine length = 25 m, height = 1.25 m, mesh size [arms] = 0.5–1 cm, mesh size [bag] = 0.3 cm) in August and September. The seine was deployed from a boat over the meadow and dragged onto the beach. During each sampling, the seine was dragged once parallel and once perpendicular to the shoreline covering an area of approximately 250 m^2. We identified all individuals to species and measured their lengths. To complement the stable isotope analysis (see below), we also analyzed the stomach contents of the most abundant fish species caught in the gill nets in June, July, August, and September (perch *Perca fluviatilis*, roach *Rutilus rutilus*, and Baltic herring *Clupea harengus membras*). Stomachs were removed after capture, and stomach contents identified to the lowest possible level.

At Fårö in 2012, we sampled fish predators using Nordic gillnets, deployed once before the field experiment during a 12 h cycle (overnight) in August. We also used minnow traps (45 × 22 cm, 25 mm openings, mesh size = 3 mm) to catch small intermediate predators. These traps were deployed twice (five traps each time) for 24 h in August.

Table 1. Environmental characteristics in the two study sites. Data from Boström et al. 2006 and Röhr et al. 2016. Wave exposure was calculated based on the simplified wave exposure model (Isæus and Rygg, 2005). Sediment and eelgrass characteristics are given at mean ± SE.

Site	Mean fetch (km)	Max fetch (km), direction	Wave exposure (m^2 s^{-1})	Dominant sediment type	Silt content (%)	Sediment organic content (%)	Shoot density (m^{-2})	Above-ground biomass (g dw m^{-2})	Below-ground biomass (g dw m^{-2})
Ängsö	1.9	15, SW	11,274	Medium sand	6.3 ± 0.5	0.20 ± 0.10	604 ± 98	91 ± 6	63 ± 9
Fårö	3.6	36, NE	12,322	Fine sand	6.3 ± 0.5	0.20 ± 0.10	304 ± 32	138 ± 20	167 ± 28

Table 2. Species and material used in the stable isotope analysis. All sampled material was collected from the eelgrass meadow at Ängsö in June–July 2011.

Group	Species/description
Detritus	Detritus from the sediment surface
Algae	*Chara* spp., *Cladophora glomerata*, *Fucus vesiculosus*, *Polysiphonia spp.*, *Ulva spp.*, epiphytes scraped from eelgrass leaves, drift algae
Angiosperms	*Potamogeton perfoliatus*, *Stuckenia pectinata*, *Zannichellia palustris*, fresh *Zostera marina*, decaying *Z. marina*
Bivalves	*Cerastoderma glaucum*, *Limecola balthica*, *Mya arenaria*, *Mytilus trossulus*
Gastropods	Hydrobiidae (*Ecrobia ventrosa*, *Peringia ulvae*), *Theodoxus fluviatilis*
Amphipods	*Gammarus locusta*, *G. oceanicus*, *G. salinus*, *G. zaddachi*
Decapods	*Crangon crangon*, *Palaemon adspersus*
Isopods	*Idotea balthica*, *I. chelipes*
Polychaetes	*Hediste diversicolor*
Insects	Chironomidae (larvae)
Fish	*Abramis brama*, *Clupea harengus membras*, *Gasterosteus aculeatus*, *Nerophis ophidion*, *Perca fluviatilis* (9, 12, 20, 22 and 24 cm size classes), *Pomatoschistus minutus*, *Pungitius pungitius*, *Sygnathus typhle*, *Rutilus rutilus* (10 cm and 20 cm size classes)

Stable isotope analysis

We used stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis to determine the structure of the food web at Ängsö in 2011. Samples ($n = 5$–10 per species) of the main primary producers and consumers were collected in June–July during the fish and invertebrate field surveys. Species included in this analysis included angiosperms, macroalgae, epiphytes, drift algae, detritus, invertebrates, and fish (Table 2).

For the angiosperms and algae, we took samples from the aboveground leaf and thallus tissue, respectively. For the fish, decapods, and bivalves we took samples from the muscle tissue while for the other invertebrates we used the whole individual. The samples were dried (48 h at 60°C) immediately after collection, and then ground to a fine powder. The stable isotope analysis was carried out using a continuous flow isotope ratio mass spectrometer (PDZ Europa 20-20 IRMS) at the Stable Isotope Facility of the University of California, Davis (Davis, USA), and stable isotope values are expressed as standard values relative to international standards (Vienna PeeDee Belemnite for C and atmospheric nitrogen for N). The isotope values are expressed in the δ‰ notation and were calculated as:

$$\delta^{13}\mathrm{C or} \delta^{14}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000,$$

where R is the isotope ratio of ^{13}C:^{12}C or ^{15}N:^{14}N.

Stable isotope analysis of gastropod samples included the whole animal (muscle and shell), rather than only the muscle as is recommended. Because shell inclusion highly biases $\delta^{13}C$ values (Mateo et al. 2008), we corrected these values to bring them in line with previous studies in the same area (Gagnon et al. 2013), though we acknowledge that such a generalized correction factor is not ideal and the values presented here for gastropod species should be considered estimates.

$$\mathrm{Gastropods}: \delta^{13}\mathrm{C}_{\mathrm{corrected}} = \delta^{13}\mathrm{C} - 3.00.$$

Lipid values can also bias $\delta^{13}C$ values, so we corrected the $\delta^{13}C$ values for fish as suggested by Post et al. (2007), based on the C:N ratio of the fish tissue. Values were not corrected for invertebrates, as the C:N ratio is not a good predictor of lipid content for aquatic invertebrates (Kiljunen et al. 2006).

$$\mathrm{Fish}: \delta^{13}\mathrm{C}_{\mathrm{corrected}} = \delta^{13}\mathrm{C} - 3.32 + 0.99 \times \mathrm{C}:\mathrm{N}.$$

Stable isotope mixing models

We only assessed the diet of consumers whose main food sources were likely to be included in this study (i.e., we did not analyze filter or deposit feeders, or species that mostly feed on infauna, plankton, or meiofauna). When the position of a consumer in the dual isotope plot was enclosed within the polygon drawn by its potential sources (corrected for the trophic enrichment factor [TEF]), we used stable isotope mixing models to identify the contribution of each source. Potential food sources were selected from these polygons and stomach contents analyzed in this study, as well as from expert knowledge on the ecology of these species in the Baltic Sea (Table 3). To improve precision of the estimates, we combined sources that were isotopically and functionally similar (i.e., we grouped small fish [*Gasterosteus aculeatus*, *Pomatoschistus minutus*, *Pungitius pungitius*], crustacean mesograzers [*Gammarus* spp., *Idotea* spp.), bivalves [*Cerastoderma glaucum*, *Limecola balthica*, *Mytilus trossulus*], and filamentous algae and detritus [*Cladophora glomerata*, *Polysiphonia* spp., *Ulva* spp., drift algae, detritus]). To run these analyses, we used the package "simmr" (Parnell 2019) in R version 3.6.2 (R Core Team 2019), which uses Bayesian inference to determine the potential contribution of different prey items to the diet of a consumer (see Parnell et al. 2010, 2013 for more details). The TEF values were set at 2.5 ± 2.5‰ and 0.41 ± 1.14‰ for primary consumers, and 3.23 ± 0.41‰ and 0.91 ± 1.04‰ for higher-level consumers, for $\delta^{15}N$ and $\delta^{13}C$, respectively (Vander Zanden and Rasmussen 1999).

Field experiment

Experimental design

We crossed nutrient fertilization with crustacean mesograzer reduction in a 4-week fully factorial field experiment which was first run at Ängsö in August–September 2011,

Table 3. Consumers and sources used in the diet mixing analyses. TEF values were set at 2.5 ± 2.5‰ and 0.41 ± 1.14‰ for primary consumers, and 3.23 ± 0.41‰ and 0.91 ± 1.04‰ for higher-level consumers, for $\delta^{15}N$ and $\delta^{13}C$, respectively (Vander Zanden and Rasmussen 1999).

Consumer		Sources
Higher-level consumers		
Perca fluviatilis (24 cm)	Fish	*R. rutilus* 10 cm, small fish (*G.* aculeatus + *P. pungitius* + *P. minutus*), *C. crangon*, *P. adspersus*, crustacean mesograzers (*Gammarus* spp. + *Idotea* spp.)
Perca fluviatilis (22 cm)	Fish	Same as above
Perca fluviatilis (20 cm)	Fish	Same as above
Perca fluviatilis (12 cm)	Fish	Small fish (*G.* aculeatus + *P. pungitius* + *P. minutus*), *C. crangon*, *P. adspersus*, crustacean mesograzers (*Gammarus* spp. + *Idotea* spp.)
Perca fluviatilis (9 cm)	Fish	Same as above
Rutilus rutilus (20 cm)	Fish	Hydrobiidae, *T. fluviatilis*, bivalves (*C. glaucum*, *M. balthica*, *M. trossulus*)
Rutilus rutilus (10 cm)	Fish	Same as above
Secondary consumers		
Gasterosteus aculeatus	Fish	Crustacean mesograzers (*Gammarus* spp. + *Idotea* spp.), Chironomidae
Pungitius pungitius	Fish	Same as above
Palaemon adspersus	Decapod	Filamentous algae + detritus, Chironomidae, *Gammarus* spp.
Primary consumers		
Gammarus oceanicus	Amphipod	Filamentous algae + detritus, epiphytes
Gammarus salinus	Amphipod	Same as above
Gammarus zaddachi	Amphipod	Same as above
Idotea balthica	Isopod	*F. vesiculosus*, *Z. marina*, Filamentous algae + detritus, epiphytes
Idotea chelipes	Isopod	Same as above
Hydrobiidae	Gastropod	Filamentous algae + detritus, epiphytes
Theodoxus fluviatilis	Gastropod	Same as above

then repeated at Fårö in August–September 2012 (with the exception of eelgrass growth and epibiont biomass, which were not measured at Fårö). The experimental setup was based on standardized protocols developed within the *Zostera* Experimental Network (www.zenscience.org), described in detail in Duffy et al. (2015). Prior to that global study, the methods (experimental duration, nutrient fertilization, mesograzer deterrence) were tested in detail in a pilot study (Whalen et al. 2013). In brief, we established 40 triangular plots (each side 50 cm long,) placed 2–3 m apart in two rows (5 m apart). We placed the plots at 2–4 m depth within monospecific eelgrass stands in the meadow, 3–5 m from the nearest edge to avoid edge effects. The water temperature was monitored with Onset HOBO Pendant data loggers, and varied from 14 to 20°C during the experiments.

To manipulate bottom-up and top-down processes, we used a no-cage method developed and successfully applied in eelgrass meadows around the world within the *Zostera* Experimental Network (Whalen et al. 2013; Reynolds et al. 2014; Duffy et al. 2015). For nutrient addition (i.e., modified bottom-up control, indicated as N−/N+), we attached a 1 mm mesh bag containing slow-release fertilizer (300 g fertilizer pellets, N:P:K = 14:14:14, Plantacote, SQM VITAS, Amsterdam, Netherlands TM) to a metal rod in the centre of the plot. In control treatments, we placed an empty bag in the center of the plot. To deter crustacean mesograzers (i.e., modified top-down control by crustacean mesograzers), indicated as D−/D+), we used plaster blocks containing 10% of the insecticide carbaryl (Bayer Crop Science) (Poore et al. 2009). We focused on crustacean mesograzers (isopods and amphipods), as they are effective grazers of filamentous algae and more commonly affected by trophic cascades caused by the overexploitation of top predators than gastropod mesograzers (Baden et al. 2012; Riera et al. 2020). Of the mesograzers in this system, only adult isopods *Idotea* spp. can directly graze the eelgrass itself, however it is not a preferred nor optimal food source (nkoski et al. 2009). The carbaryl plaster blocks were attached to each of the three corner poles at 20 cm above the sediment surface. In the control treatments, we attached plaster blocks without carbaryl. The plaster blocks were replaced once a week during the 4-week experimental period. We hereafter refer to these treatments as Control (D−N−), Nutrient addition (D−N+), Deterrence (D+N−), and Combined (D+N+).

Response variables

To estimate eelgrass growth at Ängsö, we marked five shoots per plot with a syringe needle 7 days prior to the end of the experiment, then measured the dry weight of the new growth at the end of the experiment (according to the plastochrone method; Short and Duarte 2001). At the end of the experiment, we also harvested all eelgrass shoots, along with all associated invertebrates and any filamentous drift algae, by enclosing the centre of each plot in a 20-cm diameter 500 μm-mesh bag. In the laboratory, we separated and analyzed the marked shoots for growth, then randomly selected five additional shoots: four of which we scraped to obtain dry epibiont biomass, and one from which we measured epiphyte chl *a* concentration (a proxy for epiphyte biomass; Duffy and Harvilicz 2001). We also separated the filamentous drift algae from the eelgrass, and measured their dry weight. Finally, we

dried the eelgrass shoots at 60°C for 48 h to obtain the total dry weight for each plot.

The invertebrates were sieved to obtain size class (sieve series: 8.0, 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, 0.7, 0.5 mm) and sorted to the lowest taxonomical level possible. We then estimated the biomass (ash-free dry weight) of all invertebrates based on their size classes using empirical equations for crustaceans, bivalves, and polychaetes (Edgar 1990). The biomass of insect larvae and other arthropods was estimated using the equation for crustaceans, while that of other annelids was estimated using the equation for polychaetes. We excluded shell-less gastropods (*Limapontia capitata*) as the equations would not properly estimate its biomass and in any case, their biomass would be relatively insignificant. We considered four taxonomic groups in the analyses (amphipods, isopods, crustaceans [amphipods + isopods + decapods], gastropods), along with an additional mesograzer functional group (amphipods + isopods + gastropods). We also calculated the total abundance of invertebrates, the species richness, the Shannon's diversity index (H'), and the Pielou's evenness index (J') of each plot. All invertebrate abundances and biomasses were standardized to eelgrass dry weight in the plot.

Statistical analysis

The experimental data were analyzed using generalized linear models in R version 3.6.2 (R Core Team 2019). We used the "glm" function in the default "stats" package, along with the "Anova" function in the "car" package to run *F*-tests (for normal and Gamma distributed factors) and χ^2 tests (for -Poisson-distributed factors). We used normal distribution for species richness, Shannon diversity, evenness, chl *a*, and eelgrass biomass, Gamma distribution for drift algae biomass, and Poisson distribution for all invertebrate abundances and biomasses. In all models, we included Deterrence, Nutrient addition, and their interaction as fixed factors. For eelgrass growth, we used a generalized linear mixed model ("lmer" function in the "lme4" package; Bates et al. 2015) with the same fixed factors (Deterrence, Nutrient addition, and their interaction), and added Plot ID as a random factor to account for the five sampled shoots per plot. When necessary, we performed post-hoc Tukey tests (using the "emmeans" package; (Lenth 2020) to determine which treatments differed significantly.

Results

Fish and invertebrate community

Gill net catches at Ängsö were dominated by perch and roach, with small numbers of Baltic herring, three-spined stickleback, and other cyprinids (Fig. S1a, A1b). Perch were more abundant during the day (07:00–19:00) while roach were more abundant at night (19:00–07:00) (Fig. S2). The beach seine catches at Ängsö were dominated by small fish, mainly sand gobies, nine-spined sticklebacks, and pipefish (*Nerophis ophidion* and *Sygnathus typhle*) (Fig. S1c). At Fårö, the gill net catch in August was dominated by perch (80%) with minor contributions (10%) of Baltic herring (Fig. S1d), while the minnow trap catch was dominated by black gobies (*Gobius niger*) and shrimp (*Palaemon adspersus* and *Crangon crangon*) (Fig. S1e).

The invertebrate community at Ängsö consisted of bivalves (*C. glaucum*, *Parvicardium hauniense*, *M. trossulus*, and *Mya arenaria*), gastropods (*Ecrobia ventrosa*, *Peringia ulvae*, and

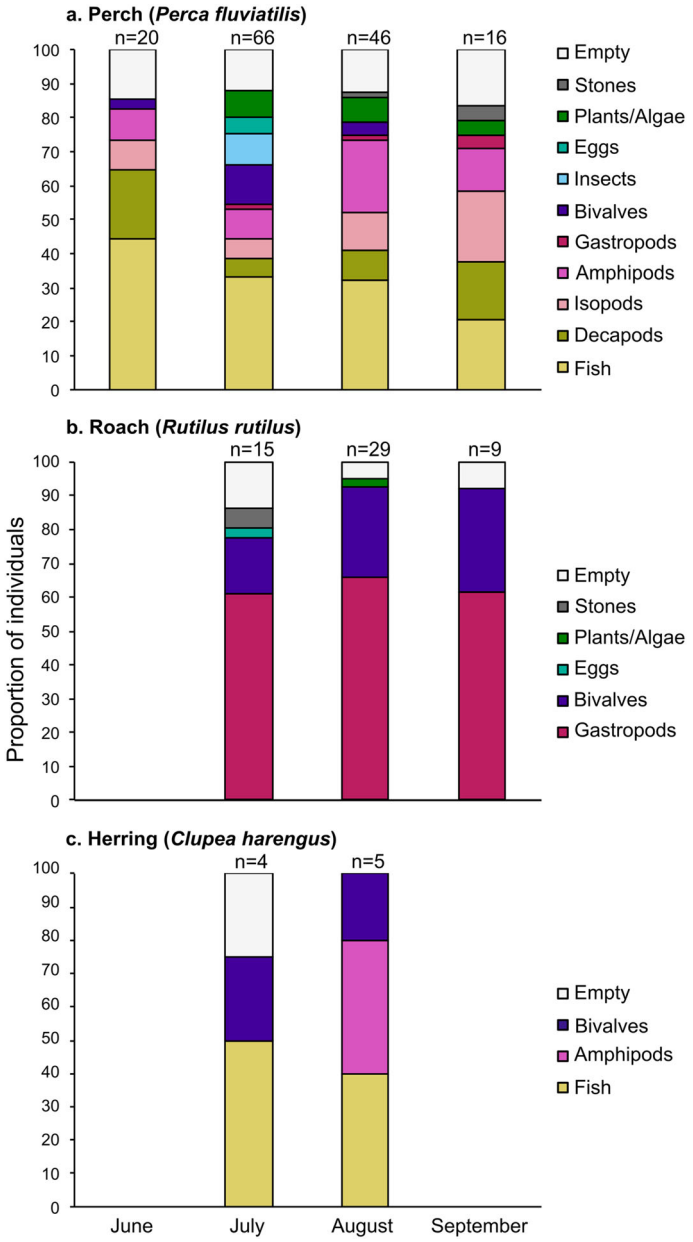

Fig 1. Proportion of individual stomachs of (**a**) perch *Perca fluviatilis*, (**b**) roach *Rutilus rutilus*, and (**c**) Baltic herring *Clupea harengus membras* containing different prey items. Individuals were caught in gill nets at Ängsö in June, July, August, and September 2011. *N* = number of individuals sampled for each species and timepoint. No roach were sampled in June, and no herring in June or September.

Theodoxus fluviatilis), crustacean mesograzers (amphipods *Gammarus* spp., isopods *Idotea* spp. and *Jaera albifrons*, and Chironomid larvae (Table S1). At Fårö, invertebrate densities were overall much higher than at Ängsö, but the community was composed of mostly the same species (Table S1): bivalves (*C. glaucum* and *M. trossulus*), gastropods (*E. ventrosa*, *P. ulvae*, *L. capitata*, and *T. fluviatilis*), and crustacean mesograzers (amphipods *Gammarus* spp. and isopods *Idotea* spp. and *J. albifrons*), and insect larvae (Chironomidae and Trichoptera).

Stomach contents and food web structure

In June, nearly 50% of perch individuals had fed on fish, and this proportion decreased throughout the summer to ~ 20% in September, while the contribution of insects and crustaceans (decapods, isopods, and amphipods) to the diet increased from 30% to 40% of individuals in June–August to 50% in September (Fig. 1a). Roach fed almost exclusively on gastropods (60%–70%) and bivalves (20%–30%) throughout the summer (Fig. 1b). There were few Baltic herring specimens analyzed, and their diet consisted of fish (juveniles of unidentified species), bivalves, and amphipods (Fig. 1c).

The dual stable isotope plot (Fig. 2), showed four distinct trophic levels – primary producers (angiosperms and algae), primary consumers (bivalves, insect larvae, polychaetes, amphipods, isopods), secondary consumers (shrimp, fish), and tertiary consumers (piscivorous fish). The primary producers clustered into three groups according to $\delta^{13}C$ signatures: larger macrophytes (angiosperms, *Fucus vesiculosus*, and charophytes) with the highest $\delta^{13}C$ values, epiphytic algae with intermediate $\delta^{13}C$ values, and filamentous algae (drift algae, *C. glomerata*, *Polysiphonia* spp., and *Ulva* spp.) with the lowest $\delta^{13}C$ values. The stable isotope signature of detritus overlapped with the latter group, indicating that most of the detritus was of filamentous algal origin. The primary consumers were clustered into two distinct groups according to $\delta^{13}C$ signatures: the filter feeding bivalves (*C. glaucum*, *L. balthica*,

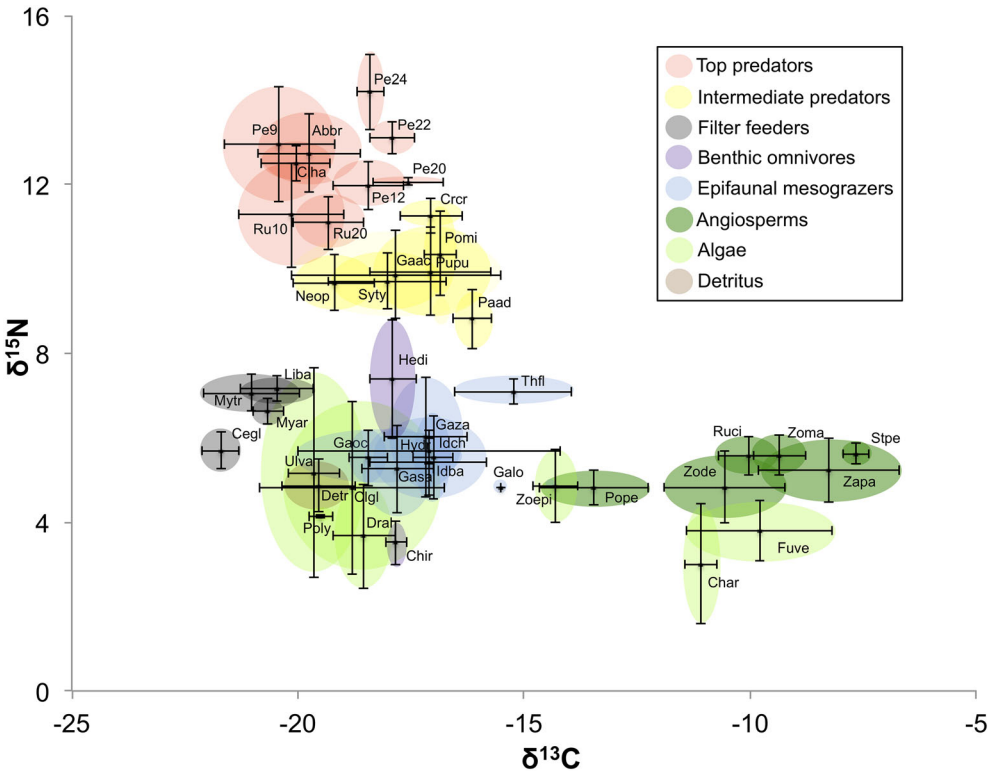

Fig 2. Dual isotope plot ($\delta^{13}C$ and $\delta^{15}N$) of the food web in the Ängsö eelgrass meadow. See Table 2 for description of species and material used. Species abbreviations are listed below in alphabetical order by subgroup. Numbers after the perch (Pe) and roach (Ru) abbreviations indicate the size class in cm. Top-predators: Abbr, *Abramis brama*; Clha, *Clupea harengus membras*; Pe, *Perca fluviatilis*; Ru, *Rutilus rutilus*. Intermediate predators: Crcr, *Crangon crangon*; Gaac, *Gasterosteus aculeatus*; Neop, *Nerophis ophidion*; Paad, *Palaemon adspersus*; Pomi, *Pomatoschistus minutus*; Pupu, *Pungitius pungitius*; Syty, *Sygnathus typhle*. Filter feeders: Cegl, *Cerastoderma glaucum*; Liba, *Limecola balthica*; Myar, *Mya arenaria*; Mytr, *Mytilus trossulus*. Benthic omnivores: Chir, Chironomid larvae; Hedi, *Hediste diversicolor*. Epifaunal mesograzers: Gaoc, *Gammarus oceanicus*; Gasa, *Gammarus salinus*; Gaza, *Gammarus zaddachi*; Idba, *Idotea balthica*; Idch, *Idotea chelipes*; Hydr, Hydrobiidae (*Ecrobia ventrosa*, *Peringia ulvae*); Thfl, *Theodoxus fluviatilis*. Angiosperms: Pope, *Potamogeton perfoliatus*; Ruci, *Ruppia cirrhosa*; Stpe, *Stuckenia pectinata*; Zapa, *Zannichellia palustris*; Zode, decaying *Zostera marina*; Zoma, fresh *Zostera marina*. Algae: Char, *Chara* spp.; Clgl, *Cladophora glomerata*; Dral, Drift algae; Fuve, *Fucus vesiculosus*; Poly, *Polysiphonia* spp.; Ulva, *Ulva* spp.; Zoepi, *Zostera marina* epiphytes. Detr, Detritus. Note that neither phytoplankton nor particulate organic matter (POM), i.e., major food sources for filter feeders, were sampled in this study, but typically have $\delta^{13}C$ values of − 18 to − 20 and − 25 to − 30, respectively (Gagnon et al. 2013).

M. arenaria, *M. trossulus*) were the most depleted group, with $\delta^{13}C$ values closest to those typical of phytoplankton (– 18 to – 20‰, Rolff et al. 2000; Fig. 2), while the crustacean and gastropod mesograzers along with chironomid larvae and the polychaete *Hediste diversicolor* had comparatively high $\delta^{13}C$ values (14 to – 17‰; Fig. 2).

The SI mixing models supported the stomach content analysis showing that perch were an important predator of small fish. The proportion of fish in the diet increased with perch size class, with the largest size classes feeding on increasingly larger fish such as roach (Fig. 3). Both size classes of roach fed primarily on bivalves and gastropods (Fig. 3). Mesopredatory sticklebacks and the shrimp *P. adspersus* fed primarily on crustacean mesograzers (amphipods and/or isopods). The SI mixing models also showed that amphipods, isopods, and gastropods fed on ~ 75% filamentous algae and detritus, with the remaining proportion of the diet composed of epiphytic algae (and larger macrophytes in the case of isopods; Fig. 3). Accordingly, the Ängsö eelgrass food web showed three main pathways of energy flow: one with enriched $\delta^{13}C$ values based on algae and epiphytes and passing through crustacean mesograzers to sticklebacks and perch, another also based on algae and epiphytes passing through gastropods and roach, and a ^{13}C-depleted pathway based on POM sources (though not measured in this study, these are usually ^{13}C-depleted with values between – 25 and – 30‰; e.g., Gagnon et al. 2013), passing from filter-feeding bivalves to herring and roach (Figs. 1, 2).

Field experiment

Faunal responses

The interaction of experimental nutrient addition and crustacean mesograzer deterrence treatments had significant effects on total invertebrate abundance and biomass, but these effects differed between the two study sites. At Ängsö, deterrence modified the effects of nutrient addition: nutrient addition led to a two-fold increase of invertebrate abundance when crustacean mesograzers were present, while it had no effect when they were excluded (Fig. 4a). Deterrence also moderated the effects of nutrient addition on biomass, but to a lesser extent (Fig. 5a). Conversely, at Fårö, nutrient addition instead led to a 25% reduction in both invertebrate abundance and biomass when crustacean mesograzers were present, but invertebrate abundance and biomass were approximately 50% higher in the deterrence treatments with

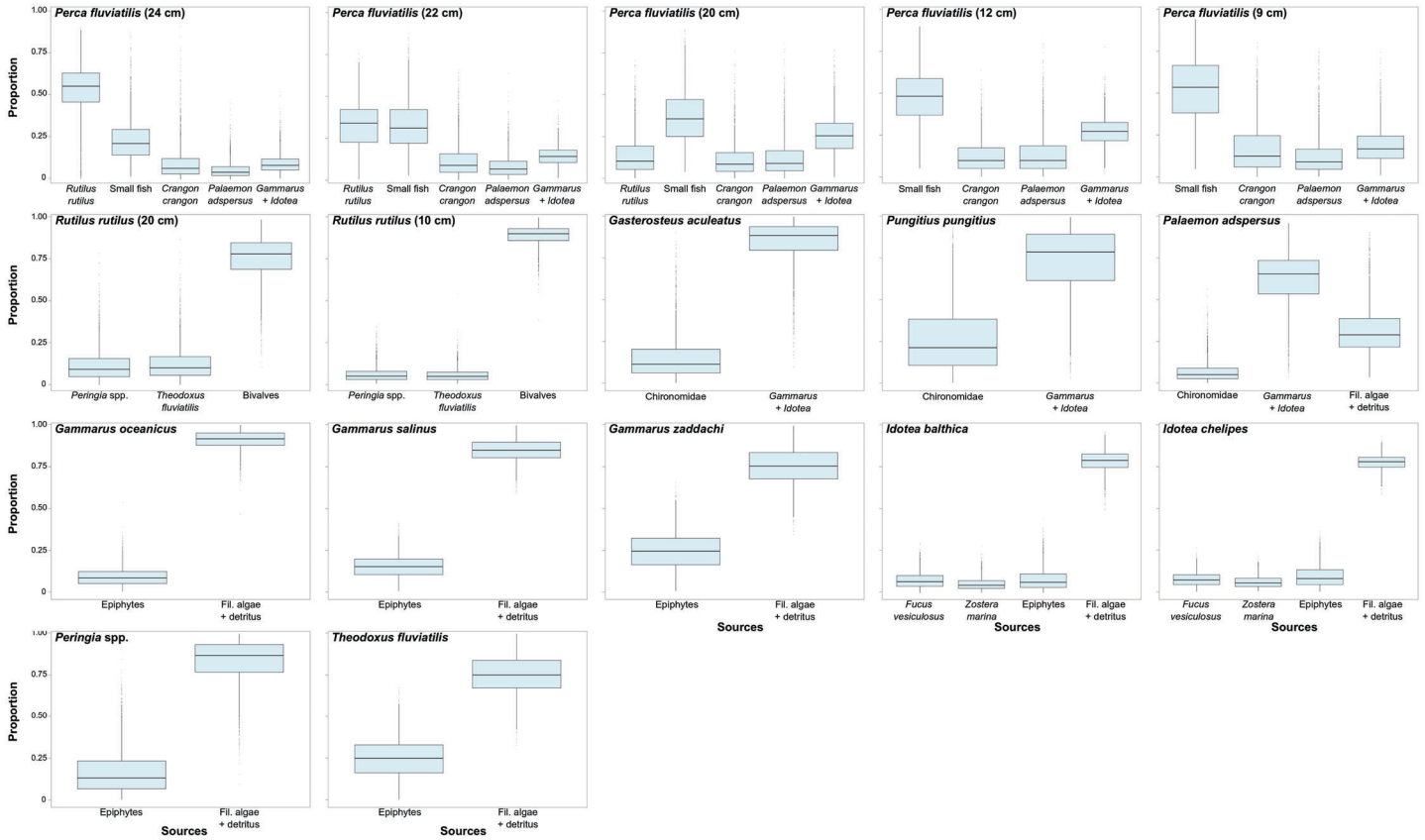

Fig 3. Results of SI mixing models (see Table 3 for details on the sources used). Top row = piscivorous fish, second row = mesopredatory fish and shrimp, third row = crustacean mesograzers, bottom row = gastropod mesograzers. Boxes represent median ± 25% confidence intervals, and error bars represent 95% confidence intervals. Outliers are shown as single points.

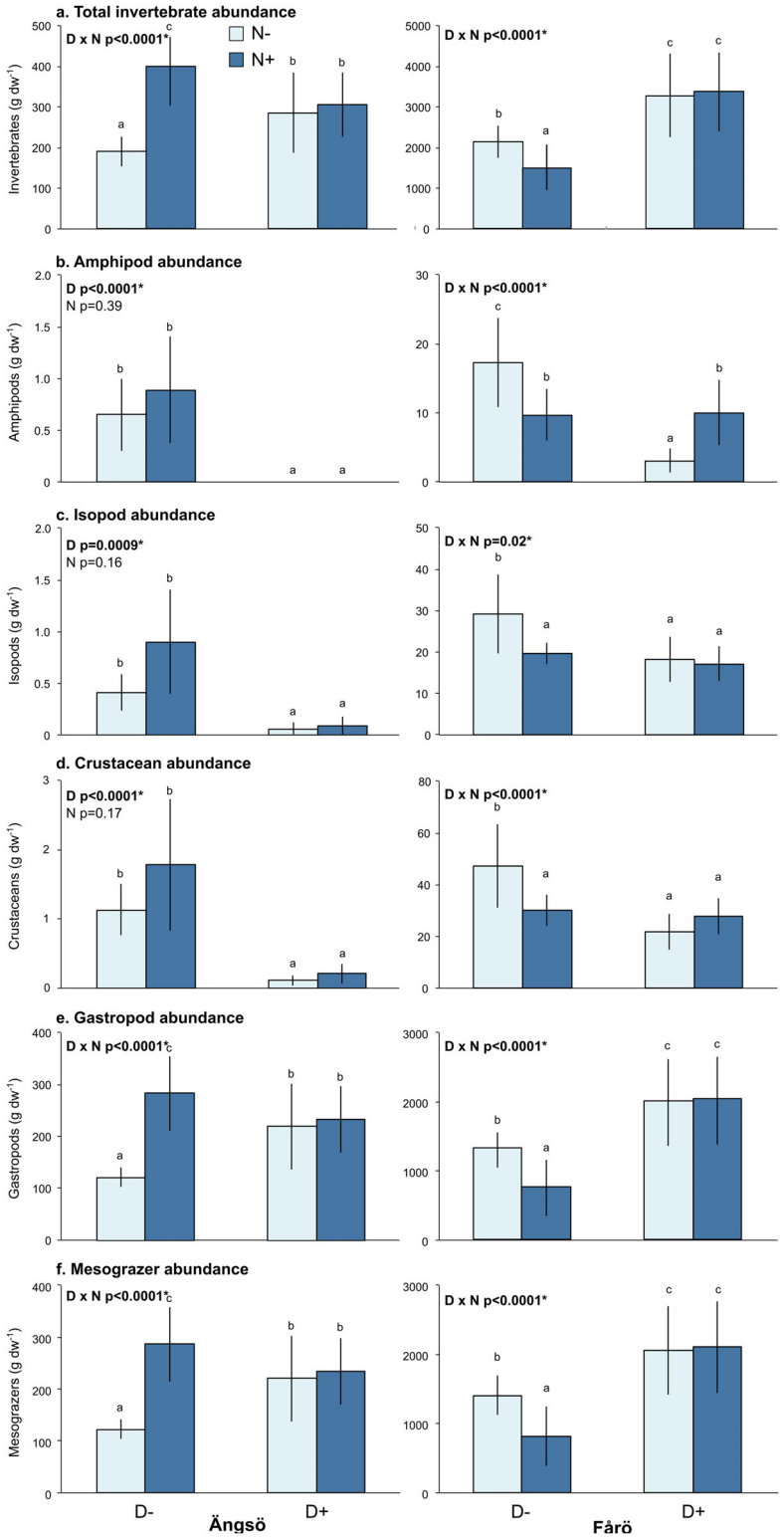

Fig 4. Invertebrate abundance (mean ± SE) in the field experiment: (**a**) total invertebrates, (**b**) amphipods, (**c**) isopods, (**d**) crustaceans, (**e**) gastropods, and (**f**) mesograzers. Crustaceans include amphipods and isopods, while mesograzers include amphipods, isopods, and gastropods. N + =nutrient addition and D + =crustacean mesograzer deterrence. Note the differing *y*-axis scales between sites. Significant terms are denoted in bold with an asterisk, and different letters above bars indicate significant differences between treatments ($p < 0.05$). See Table S2 for full statistical results.

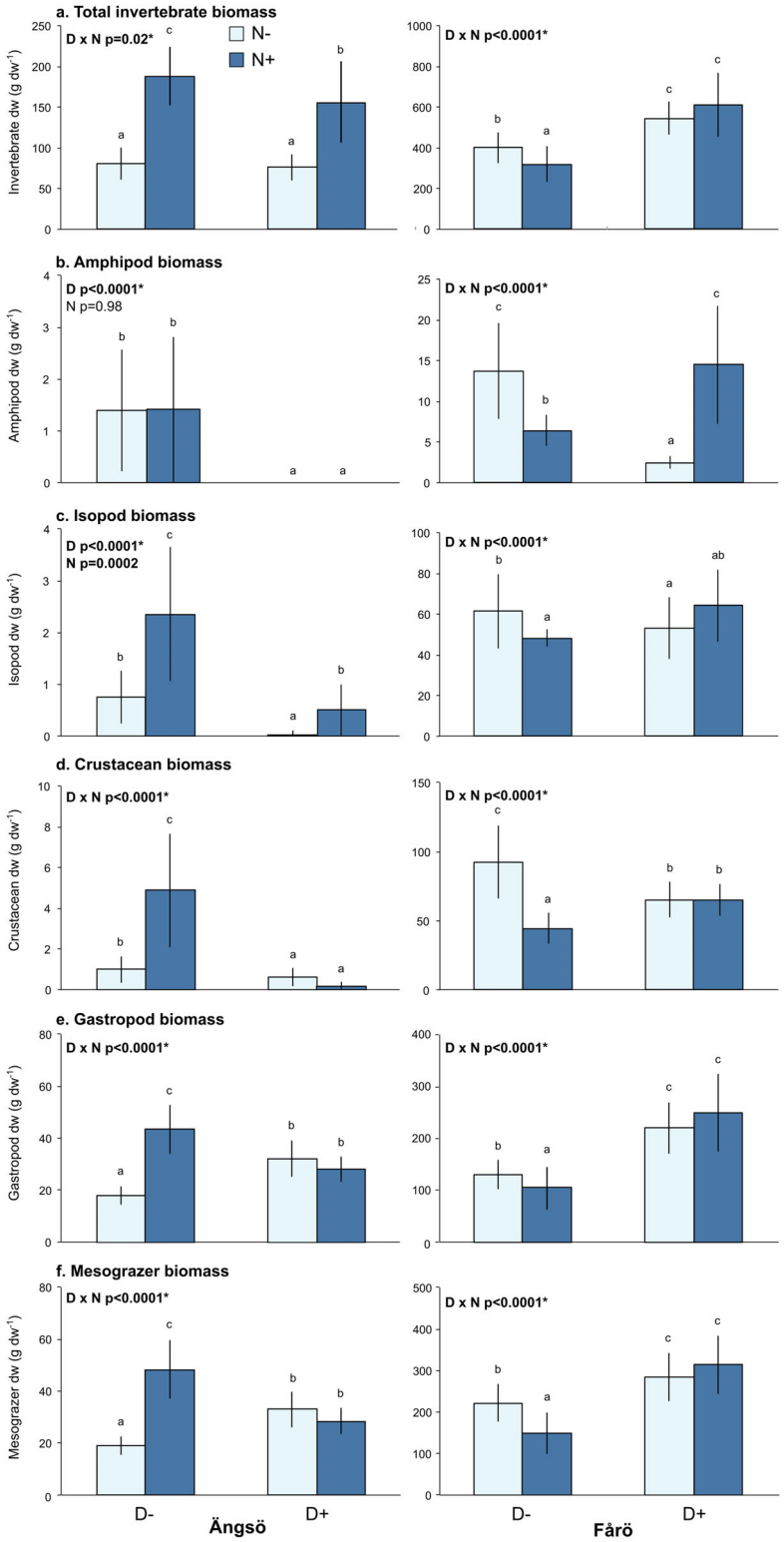

Fig 5. Invertebrate biomass (estimated ash-free dry weight, mean ± SE) in the field experiment: (**a**) total invertebrates, (**b**) amphipods, (**c**) isopods, (**d**) crustaceans, (**e**) gastropods, and (**f**) mesograzers. Crustaceans include amphipods and isopods, while mesograzers include amphipods, isopods, and gastropods. N + =nutrient addition and D + =crustacean mesograzer deterrence. Note the differing y-axis scales between sites. Significant terms are denoted in bold with an asterisk, and different letters above bars indicate significant differences between treatments ($p < 0.05$). See Table S2 for full statistical results.

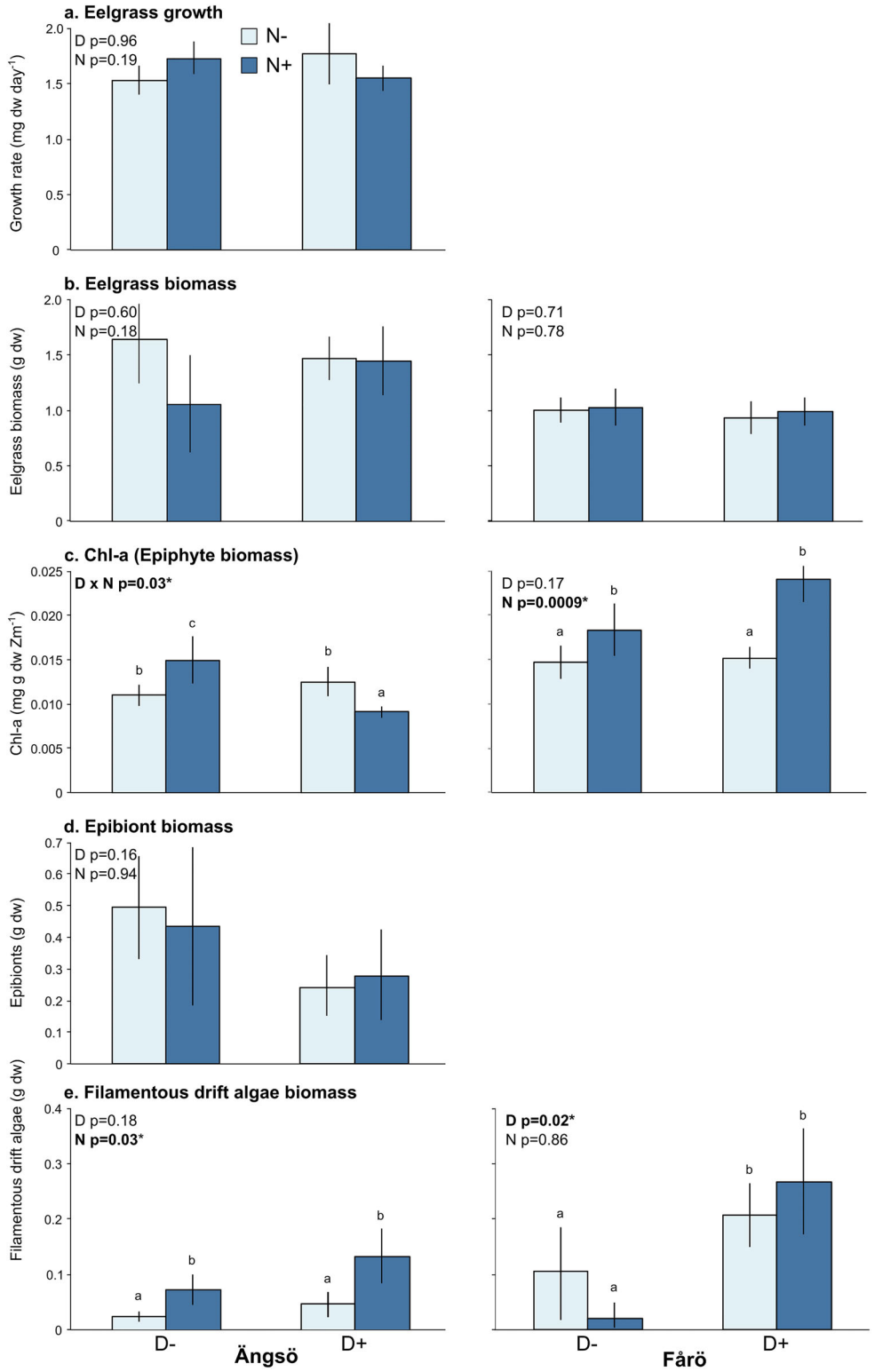

Fig 6. Algal and plant responses (mean ± SE) in the field experiment: (**a**) eelgrass growth, (**b**) eelgrass biomass, (**c**) chl *a* (epiphyte abundance), (**d**) epibiont biomass, and (**e**) filamentous drift algae biomass at Ängsö (left panel) and Fårö (right panel). Neither eelgrass growth nor epibiont biomass were measured at Fårö 2012. N + = nutrient addition and D + = crustacean mesograzer deterrence. Significant terms are denoted in bold with an asterisk. Significant terms are denoted in bold with an asterisk, and different letters above bars indicate significant differences between treatments ($p < 0.05$). See Table S3 for full statistical results.

and without nutrient addition (Figs. 4a, 5a). Though we did not directly compare the two sites, the total abundance and biomass of invertebrates in the control plots were approximately an order of magnitude higher at Fårö than Ängsö.

At Ängsö, the abundance and biomass of isopods, amphipods, and total crustaceans was nearly zero in the deterrence treatments (Table S2) indicating that the carbaryl method was successful at this site (Figs. 4b–d, 5b–d). Nutrient addition also had a positive effect on isopod and crustacean biomass (Fig. 5c, d). However, at Fårö deterrence and nutrients had significant interactive effects on the abundance and biomass of these species (Table S2): without added nutrients, deterrence reduced these species by 20%–50% but with nutrients, deterrence either increased their abundance/biomass (amphipods) or had no addition effect (isopods, crustaceans; Fig. 4b–d, 5b–d). Deterrence and nutrients also had significant interactive effects on the abundance and biomass of gastropods and total mesograzers at both sites (Table S2). At Ängsö, nutrient addition alone led to a two-fold increase in the abundance and biomass of these groups, while the deterrence treatment led to a similar increase with no additional effect of nutrient addition (Fig. 4e,f, 5e,f). At Fårö, nutrient addition instead led to a ~ 50% decrease in abundance and biomass, while deterrence (with or without nutrients) led to a ~ 30% increase (Fig. 4e,f, 5e,f).

Species richness, Shannon diversity, and evenness at Ängsö were consistent across treatments, as were species richness and evenness at Fårö (Table S3; Fig. S3). At Fårö nutrient addition had a slight (~ 10%) positive effect on Shannon diversity, while deterrence had a slight (~ 5%) negative effect, with no interaction (Table S3; Fig. S3).

Plant and algae responses

At Ängsö, neither nutrient addition nor deterrence had any effects on eelgrass growth and biomass (Table S3; Fig. 6a,b). There was, however, a significant effect of their interaction (Table S3) on eelgrass epiphyte biomass: nutrient addition led to ~ 20% increase biomass when crustacean mesograzers were present, but a ~ 20% decrease when they were excluded (Fig. 6c). There were no significant effects on epibiont biomass (Table S3; Fig. 6d), while nutrient addition resulted in a greater than two-fold increase of drift algal biomass in both the presence and absence of crustacean mesograzers (Table S3; Fig. 6e). At Fårö in 2012, neither nutrient addition nor deterrence had significant effects on eelgrass biomass (Table S3; Fig. 6b). Nutrient addition led to a ~ 30%–40% increase in epiphyte biomass both in the presence and absence of crustacean mesograzers (Table S3; Fig. 6c). Mesograzer deterrence led to a strong increase in filamentous drift algae biomass both with and without nutrient addition (Fig. 6e).

Discussion

In this study, we assessed food web interactions in eelgrass communities in the northern Baltic Sea by combining fish diet and stable isotope analyses, along with field experiments. Firstly, we were able to determine the most relevant linkages in the eelgrass trophic network, and confirm three main trophic pathways based on: (1) epiphytes and filamentous algae passing through crustacean mesograzers to sticklebacks to perch, (2) epiphytes and filamentous algae passing through gastropod mesograzers to roach, and (3) POM sources passing through filter feeding bivalves to herring and roach, all of which converge at the highest trophic levels as predatory fish feed on other fish. The first pathway in particular supports the potential for trophic cascades to occur in northern Baltic eelgrass meadows, as has been shown in other northern European eelgrass meadows (Moksnes et al. 2008; Baden et al. 2012). Second, we studied how nutrient addition and crustacean mesograzer deterrence affected community structure of eelgrass communities using field experiments. We expected that eelgrass biomass and growth would be highest in unmanipulated (i.e., control) treatments and lowest in treatments that combined nutrient addition with deterrence of crustacean mesograzers, while epiphyte and filamentous drift algae biomass should follow the opposite pattern. This pattern should emerge from the combined negative of nutrient enrichment promoting algal growth (McGlathery 2001) and reduced grazing on algae by crustacean mesograzers (Sieben et al. 2011). Interestingly, our results did not match this pattern at either site.

At Ängsö in 2011, only filamentous drift algae abundance followed the expected result. Nutrient addition had a strong positive effect on epiphyte biomass when grazers were present, but, surprisingly, resulted in significantly lower epiphyte biomass when they were excluded. However, neither of these responses translated to effects on eelgrass growth or biomass. To understand these results mechanistically, we can consider the responses of the invertebrate community to the different treatments. Carbaryl (i.e., mesograzer deterrence) was highly effective in deterring crustacean mesograzers, but had no detectable effect on gastropods; in fact the total abundance and biomass of mesograzers was higher in the deterrence plots than in the control plots due to higher gastropod abundance and biomass, which likely benefited from reduced competition with crustaceans for food and substrate. All invertebrate groups also had strong positive responses to nutrient addition, likely in response to higher epiphyte availability and food sources of higher nutritional quality (Hemmi and Jormalainen 2002; Kraufvelin et al. 2006). These responses indicate that mesograzers are indeed able to quickly increase top-down pressure in the face of nutrient enrichment, though they were not able to counteract all nutrient effects, as evidenced by higher filamentous drift algae biomass in the combined treatment. On the other hand, epiphyte biomass was unexpectedly lowest in the combined treatment, potentially as a consequence of increased filamentous drift algae biomass, which can indirectly reduce eelgrass epiphytes by increasing shading and providing structure for grazers (Whalen et al. 2013). Despite the inability of mesograzers to

counter all nutrient effects, our results support their ability to exert enough top-down control to limit short-term negative effects on eelgrass growth and biomass.

We observed different patterns in the experiment results at Fårö in 2012, that are likely driven by much higher ambient invertebrate abundances and biomasses, which resulted in the deterrence treatment being less effective at deterring crustaceans at this site (only a ~ 40% reduction compared to control plots). Similarly to Ängsö, gastropod abundance and biomass also increased in the grazer exclusion plots at Fårö, possibly due to lower competition. Unlike at Ängsö, however, nutrient addition at Fårö caused a ~ 40% decrease in the abundance and biomass of all invertebrate groups without deterrence, but little effect with deterrence in all groups except amphipods. Additionally, because mesograzer abundance and biomass in the control plots were an order of magnitude higher than at Ängsö, these responses may indicate stronger ambient top-down control of algal biomass. This was supported by the observed increase in filamentous drift algae biomass but not epiphyte biomass, in response to deterrence. The factors that drive the difference in mesograzer abundance between the two sites are unclear, as the environmental conditions and eelgrass biomass are similar at both sites, though they might reflect temporal variation between the years and/or variation in other abiotic and biotic variables that were not considered here. Despite these differences between the sites, neither nutrient addition, nor carbaryl, affected eelgrass biomass over a 4-week time period.

Our results support a previous large-scale study showing that neither nutrient addition nor grazer or fish exclusion affected eelgrass growth in the northern Baltic Sea (Baden et al. 2010). Although we detected some positive effects of nutrient addition on epiphyte (in both sites) and filamentous drift algae biomass (at Ängsö), these did not translate to any effects on eelgrass. It is possible that the comparatively high mesograzer abundance, especially isopods and amphipods, is able to control most, but not all, of the increased algal biomass caused by nutrient addition as suggested by Baden et al. (2010). However, here we still found no effects on eelgrass when specifically excluding these crustacean mesograzers. We suggest that, in their absence, the role of gastropods in controlling algal biomass became more important, as they became more abundant. As confirmed by the diet mixing models, *T. fluviatilis* feeds on epiphytes and could thus control epiphyte and periphyton growth (Jacoby 1985), while Hydrobiidae can feed on filamentous algae as well as on epiphytes and detritus particles (Casagranda et al. 2005; Aberle et al. 2009). Though somewhat ignored in previous studies of Baltic eelgrass, gastropod mesograzers seem to play a key role in limiting algal biomass, and the presence of two main groups of mesograzers (i.e., crustaceans and gastropods), each of which are fed upon by separate predatory fish (perch and roach, respectively) could increase the resilience of eelgrass communities in the face of eutrophication, and to the ecosystem as a whole (Jankowska et al. 2019). As we did not explicitly test the role of gastropods by, nor is the mechanistic explanation for how they replace crustacean mesograzers clear, further research on the role of gastropods in Baltic eelgrass food web dynamics is clearly needed.

In addition to effects on the eelgrass itself, both eutrophication and trophic cascades have been linked to shifts in community assemblages in eelgrass meadows (Baden et al. 2012). In this study, we did not observe any effects on the invertebrate community as a whole (richness, diversity, or evenness), possibly due to the short time scale of the field experiment. The duration was long enough to detect eelgrass responses to shifts in nutrients and light intensity (Whalen et al. 2013), and grazers similarly react quickly to changes in their food supply. However, community shifts likely occur over multiple seasons and generations as pressures build up over time. Like many coastal seas, the Baltic Sea is undergoing rapid environmental changes (Reusch et al. 2018), and thus this study also provides a highly useful baseline of the community composition and mechanistic trophic links in Baltic eelgrass meadows. In the years following this study, filamentous algal blooms have increased at both sites (Gagnon et al. 2017), indicating that continued environmental stressors could be starting to overwhelm the resilience of these eelgrass meadows. Despite declines in perch populations across many parts of the Baltic Sea, the trend in the Finnish Archipelago Sea is towards increasing perch and roach populations (Bergström et al. 2016). While increased roach is likely to negatively impact gastropod abundances and limit their ability to control macroalgal blooms, the effects of perch are harder to predict as they feed on both mesopredatory fish and mesograzer crustaceans depending on size class. In addition to increased algal blooms, eelgrass meadows in the northern Baltic Sea have recently been subject to the introduction of novel mesopredatory species (Gagnon and Boström 2016) and heat waves (Salo et al. 2015; Salo and Gustafsson 2016), likely further eroding resilience.

Trophic interactions are important in maintaining seagrass community stability and increasing resilience to environmental stressors (Connolly et al. 2018; Yan et al. 2020). Here, we show that this also seems to be the case in a relatively low-diversity area, where trophic interactions in eelgrass meadows may provide resilience to nutrient enrichment in a highly eutrophicated area, though the exact mechanisms need further study. Understanding the trophic links and interspecific interactions that ensure resilience are essential for future conservation and restoration efforts of seagrass meadows in and beyond the Baltic Sea.

Conclusions

Northern Baltic eelgrass meadows are characterized by low diversity, simple trophic networks that feature three main trophic pathways. Neither experimental nutrient addition nor

the exclusion of crustacean mesograzers affected eelgrass growth or biomass, which suggests that top-down processes provided by high crustacean and gastropod mesograzer abundance may ensure resilience against eutrophication, as mesograzers are able to mitigate the impacts of nutrient enrichment by feeding on excess algal biomass. If one group of mesograzers is reduced (crustaceans) due to shifts in predation pressure, another mesograzer group (gastropods) may thus be able compensate, though further study is needed to fully understand this mechanism. Overall, food web interactions in northern Baltic eelgrass and associated communities likely provide resilience to local environmental pressures (in particular eutrophication), at least in the short term. As environmental stressors intensify, identifying the important trophic linkages and pathways that ensure this resilience is a critical step in planning effective conservation and restoration measures for these important ecosystems.

References

Aberle, N., A. M. Malzahn, J. Grey, H. Hillebrand, and K. H. Wiltshire. 2009. Altered complementary feeding strategies of the consumers *Hydrobia ulvae* and *Idotea emarginata* via passive selectivity. Helgoland. Mar. Res. **63**: 189–197. doi:10.1007/s10152-009-0148-9

Armitage, A. R., and J. W. Fourqurean. 2009. Stable isotopes reveal complex changes in trophic relationships following nutrient addition in a coastal marine ecosystem. Estuar. Coast. **32**: 1152–1164. doi:10.1007/s12237-009-9219-z

Baden, S., C. Boström, S. Tobiasson, H. Arponen, and P.-O. Moksnes. 2010. Relative importance of trophic interactions and nutrient enrichment in seagrass ecosystems: A broad-scale field experiment in the Baltic–Skagerrak area. Limnol. Oceanogr. **55**: 1435–1448. doi:10.4319/lo.2010.55.3.1435

Baden, S., A. Emanuelsson, L. Pihl, C.-J. Svensson, and P. Åberg. 2012. Shift in seagrass food web structure over decades is linked to overfishing. Mar. Ecol. Prog. Ser. **451**: 61–73. doi:10.3354/meps09585

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**: 1–48. doi:10.18637/jss.v067.i01

Bergström, L., O. Heikinheimo, R. Svirgsden, and others. 2016. Long term changes in the status of coastal fish in the Baltic Sea. Estuar. Coast. Shelf Sci. **169**: 74–84. doi:10.1016/j.ecss.2015.12.013

Bittick, S. J., M. Sutula, and P. Fong. 2018. A tale of two algal blooms: Negative and predictable effects of two common bloom-forming macroalgae on seagrass and epiphytes. Mar. Environ. Res. **140**: 1–9. doi:10.1016/j.marenvres.2018.05.018

Boström, C., S. Baden, A.-C. Bockelmann, and others. 2014. Distribution, structure and function of Nordic eelgrass (*Zostera marina*) ecosystems: Implications for coastal management and conservation. Aquat. Conserv. Mar. Freshw. Ecosyst. **24**: 410–434. doi:10.1002/aqc.2424

Boström, C., K. O'Brien, C. Roos, and J. Ekebom. 2006. Environmental variables explaining structural and functional diversity of seagrass macrofauna in an archipelago landscape. J. Exp. Mar. Biol. Ecol. **335**: 52–73. doi:10.1016/j.jembe.2006.02.015

Burkholder, J. M., D. A. Tomasko, and B. W. Touchette. 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. **350**: 46–72. doi:10.1016/j.jembe.2007.06.024

Casagranda, C., C. F. Boudouresque, and P. Francour. 2005. Abundance, population structure and production of *Hydrobia ventrosa* (Gastropoda: Prosobranchia) in a Mediterranean brackish lagoon, Lake Ichkeul. Tunisia. Arch. Hydrobiol. **164**: 411–428. doi:10.1127/0003-9136/2005/0164-0411

Connolly, R. M., E. L. Jackson, P. I. Macreadie, P. S. Maxwell, and K. R. O'Brien. 2018. Seagrass dynamics and resilience, p. 197–212.*In* A. Larkum, G. Kendrick, and P. Ralph [eds.], Seagrasses of Australia. Springer.

de los Santos, C. B., D. Krause-Jensen, T. Alcoverro, and others. 2019. Recent trend reversal for declining European seagrass meadows. Nat. Commun. **10**: 3356. doi:10.1038/s41467-019-11340-4

Duffy, J. E., and A. M. Harvilicz. 2001. Species-specific impacts of grazing amphipods in an eelgrass-bed community. Mar. Ecol. Prog. Ser. **223**: 201–211. doi:10.3354/meps223201

Duffy, J. E., P. L. Reynolds, C. Boström, and others. 2015. Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach. Ecology **18**: 696–705. doi:10.1111/ele.12448

Edgar, G. J. 1990. The use of size structure of benthic macrofaunal communities to estimate faunal biomass and secondary production. J. Exp. Mar. Biol. Ecol. **137**: 195–214.

Gagnon, K., and C. Boström. 2016. Habitat expansion of the Harris mud crab *Rhithropanopeus harrisii* (Gould, 1841) in the northern Baltic Sea: Potential consequences for the eelgrass food web. Bioinvasions Rec. **5**: 101–106. doi:10.3391/bir.2016.5.2.07

Gagnon, K., M. Gräfnings, and C. Boström. 2017. Shifts in coastal fish communities: Is eutrophication always beneficial for sticklebacks? Estuar. Coast. Shelf Sci. **198**: 193–203. doi:10.1016/j.ecss.2017.08.047

Gagnon, K., E. Rothäusler, A. Syrjänen, M. Yli-Renko, and V. Jormalainen. 2013. Seabird guano fertilizes Baltic Sea littoral food webs. PLoS One **8**: e61284. doi:10.1371/journal.pone.0061284

Granö, O., M. Roto, and L. Laurila. 1999. Environment and land use in the shore zone of the coast of Finland. Publ. Inst. Geogr. Univ. Turkuensis. Publ. Inst. Geogr. Univ. Turkuensis **160**: 1–76.

Gustafsson, C., and C. Boström. 2011. Biodiversity influences ecosystem functioning in aquatic angiosperm communities. Oikos **120**: 1037–1046. doi:10.2307/23015006

Gustafsson, C., and C. Boström. 2014. Algal mats reduce eelgrass (*Zostera marina* L.) growth in mixed and monospecific meadows. J. Exp. Mar. Biol. Ecol. **461**: 85–92. doi:10.1016/j.jembe.2014.07.020

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. Am. Nat. **94**: 421–425. doi:10.1086/282146

Hauxwell, J., J. McClelland, P. J. Behr, and I. Valiela. 1998. Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. Estuaries **21**: 347–360. doi:10.2307/1352481

Heck, K. L., Jr., J. R. Pennock, J. F. Valentine, L. D. Coen, and S. A. Sklenar. 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: An experimental assessment. Limnol. Oceanogr. **45**: 1041–1057. doi:10.4319/lo.2000.45.5.1041

Hemmi, A., and V. Jormalainen. 2002. Nutrient enhancement increases performance of a marine herbivore via quality of its food alga. Ecology **83**: 1052–1064. doi:10.2307/3071913

Hillebrand, H., D. S. Gruner, E. T. Borer, and others. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. Proc. Natl. Acad. Sci. USA **104**: 10904–10909. doi:10.1073/pnas.0701918104

Hoshika, A., M. J. Sarker, S. Ishida, Y. Mishima, and N. Takai. 2006. Food web analysis of an eelgrass (*Zostera marina* L.) meadow and neighbouring sites in Mitsukuchi Bay (Seto Inland Sea, Japan) using carbon and nitrogen stable isotope ratios. Aquat. Bot. **85**: 191–197. doi:10.1016/j.aquabot.2006.03.005

Howard, J. L., A. Perez, C. C. Lopes, and J. W. Fourqurean. 2016. Fertilization changes seagrass community structure but not blue carbon storage: Results from a 30-year field experiment. Estuar. Coast. **39**: 1422–1434. doi:10.1007/s12237-016-0085-1

Hughes, A. R., K. J. Bando, L. F. Rodriguez, and S. L. Williams. 2004. Relative effects of grazers and nutrients on seagrasses: A meta-analysis approach. Mar. Ecol. Prog. Ser. **282**: 87–99. doi:10.3354/meps282087

Isæus, M., and B. Rygg. 2005. Wave exposure calculations for the Finnish coast. RAPPORT LNR 5075-2005. RAPPORT LNR 5075-2005 NIVA, Norwegian Institute for Water Research

Jacoby, J. M. 1985. Grazing effects on periphyton by *Theodoxus fluviatilis* (Gastropoda) in a lowland stream. J. Freshwater Ecol. **3**: 265–274. doi:10.1080/02705060.1985.9665115

Jakobsson-Thor, S., G. B. Toth, J. Brakel, A. C. Bockelmann, and H. Pavia. 2018. Seagrass wasting disease varies with salinity and depth in natural *Zostera marina* populations. Mar. Ecol. Prog. Ser. **587**: 105–115. doi:10.3354/meps12406

Jakobsson-Thor, S., G. B. Toth, and H. Pavia. 2019. Seagrass wasting disease along a naturally occurring salinity gradient. Mar. Ecol. Prog. Ser. **614**: 67–77. doi:10.3354/meps12911

Jankowska, E., L. N. Michel, G. Lepoint, and M. Włodarska-Kowalczuk. 2019. Stabilizing effects of seagrass meadows on coastal water benthic food webs. J. Exp. Mar. Biol. Ecol. **510**: 54–63. doi:10.1016/j.jembe.2018.10.004

Jephson, T., P. Nyström, P.-O. Moksnes, and S. P. Baden. 2008. Trophic interactions in *Zostera marina* beds along the Swedish coast. Mar. Ecol. Prog. Ser. **369**: 63–76. doi:10.3354/meps07646

Kiljunen, M., J. Grey, T. Sinisalo, C. Harrod, H. Immonen, and R. I. Jones. 2006. A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotope mixing models. J. Appl. Ecol. **43**: 1213–1222. doi:10.1111/j.1365-2664.2006.01224.x

Kraufvelin, P., S. Salovius, H. Christie, F. E. Moy, R. Karez, and M. F. Pedersen. 2006. Eutrophication-induced changes in benthic algae affect the behaviour and fitness of the marine amphipod *Gammarus locusta*. Aquat. Bot. **84**: 199–209. doi:10.1016/j.aquabot.2005.08.008

Lenth, R. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.4. https://CRAN.R-project.org/package=emmeans.

Mateo, M. A., O. Serrano, L. Serrano, and R. H. Michener. 2008. Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: Implications for food web studies using stable isotopes. Oecologia **157**: 105–115. doi:10.1007/s00442-008-1052-8

McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. J. Phycol. **37**: 453–456. doi:10.1046/j.1529-8817.2001.037004453.x

Moksnes, P.-O., M. Gullström, K. Tryman, and S. Baden. 2008. Trophic cascades in a temperate seagrass community. Oikos **117**: 763–777. doi:10.1111/j.0030-1299.2008.16521.x

Muehlstein, L. K. 1989. Perspectives on the wasting disease of eelgrass *Zostera marina*. Dis. Aquat. Organ. **7**: 211–221. doi:10.3354/dao007211

Nordlund, L. M., E. W. Koch, E. B. Barbier, and J. C. Creed. 2016. Seagrass ecosystem services and their variability across genera and geographical regions. PLoS ONE **11**: e0163091. doi:10.1371/journal.pone.0163091

Orth, R. J., T. J. B. Carruthers, W. C. Dennison, and others. 2006. A global crisis for seagrass ecosystems. Bioscience **56**: 987–996 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2

Östman, Ö., J. Eklöf, B. K. Eriksson, J. Olsson, P.-O. Moksnes, and U. Bergström. 2016. Top-down control as important as eutrophication effects in North Atlantic coastal ecosystems. J. Appl. Ecol. **53**: 1138–1147. doi:10.1111/1365-2664.12654

Parnell, A. 2019. simmr: a stable isotope mixing model. R package version 0.4.1. https://CRAN.R- project.org/package=simmr.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: Coping with too much variation. PLoS One **5**: e9672. doi:10.1371/journal.pone.0009672

Parnell, A. C., D. L. Phillips, S. Bearhop, and others. 2013. Bayesian stable isotope mixing models. Environ. **24**: 387–399. doi:10.1002/env.2221

Poore, A. G. B., A. H. Campbell, and P. D. Steinberg. 2009. Natural densities of mesograzers fail to limit growth of macroalgae or their epiphytes in a temperate algal bed. Journal of Ecology. **97**: 164–175. doi:10.1111/j.1365-2745.2008.01457.x

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montaña. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia. **152**: 179–189. doi:10.1007/s00442-006-0630-x

R Core Team. 2019. R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Reusch, T. B. H., J. Dierking, H. C. Andersson, and others. 2018. The Baltic Sea as a time machine for the future coastal ocean. Science Advances. **4**: eaar8195. doi:10.1126/sciadv.aar8195

Reynolds, P. L., J. P. Richardson, and J. E. Duffy. 2014. Field experimental evidence that grazers mediate transition between microalgal and seagrass dominance. Limnol. Oceanogr. **59**: 1053–1064. doi:10.4319/lo.2014.59.3.1053

Riera, R., J. Vasconcelos, S. Baden, L. Gerhardt, R. Sousa, and E. Infantes. 2020. Severe shifts of *Zostera marina* epifauna: Comparative study between 1997 and 2018 on the Swedish Skagerrak coast. Mar. Pollut. Bull. **158**: 111434. doi:10.1016/j.marpolbul.2020.111434

Röhr, M. E., C. Boström, P. Canal-Vergés, and M. Holmer. 2016. Blue carbon stocks in Baltic Sea eelgrass (*Zostera marina*) meadows. Biogeosciences **13**: 6139–6153. doi:10.5194/bg-13-6139-2016

Rolff, C. 2000. Seasonal variation in δ13C and δ15N of size-fractionated plankton at a coastal station in the northern Baltic proper. Marine Ecology Progress Series **203**: 47–65. doi:10.3354/meps203047

Rönnbäck, P., N. Kautsky, L. Pihl, M. Troell, T. Söderqvist, and H. Wennhage. 2007. Ecosystem goods and services from Swedish coastal habitats: Identification, valuation, and implications of ecosystem shifts. Ambio **36**: 534–544 10.1579/0044-7447(2007)36[534:EGASFS]2.0.CO;2

Salo, T., and C. Gustafsson. 2016. The effect of genetic diversity on ecosystem functioning in vegetated coastal ecosystems. Ecosystems **19**: 1429–1444. doi:10.1007/s10021-016-0014-y

Salo, T., T. B. H. Reusch, and C. Boström. 2015. Genotype-specific responses to light stress in eelgrass *Zostera marina*, a marine foundation plant. Mar. Ecol. Prog. Ser. **519**: 129–140. doi:10.3354/meps11083

Schmidt, A. L., M. Coll, and H. K. Lotze. 2017. Regional-scale differences in eutrophication effects on eelgrass-associated (*Zostera marina*) macrofauna. Estuar. Coast. **40**: 1096–1112. doi:10.1007/s12237-016-0204-z

Short, F. T., and C. M. Duarte. 2001. Methods for the measurement of seagrass growth and production, p. 155–182.*In* F. T. Short and R. G. Coles [eds.], Global Seagrass research methods. Elsevier.

Sieben, K., L. Ljunggren, U. Bergström, and B. K. Eriksson. 2011. A meso-predator release of stickleback promotes recruitment of macroalgae in the Baltic Sea. J. Exp. Mar. Biol. Ecol. **397**: 79–84. doi:10.1016/j.jembe.2010.11.020

Smale, D. A., T. Wernberg, E. C. J. Oliver, and others. 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Change **9**: 306–312. doi:10.1038/s41558-019-0412-1

Thomsen, M. S., T. Wernberg, A. H. Engelen, and others. 2012. A meta-analysis of seaweed impacts on seagrasses: Generalities and knowledge gaps. PLoS One **7**: e28595. doi:10.1371/journal.pone.0028595

Unsworth, R. K. F., L. J. McKenzie, C. J. Collier, and others. 2019. Global challenges for seagrass conservation. Ambio **48**: 801–815. doi:10.1007/s13280-018-1115-y

Vafeiadou, A.-M., P. Materatski, H. Adão, M. de Troch, and T. Moens. 2013. Food sources of macrobenthos in an estuarine seagrass habitat (*Zostera noltii*) as revealed by dual stable isotope signatures. Mar. Biol. **160**: 2517–2523. doi:10.1007/s00227-013-2238-0

Valentine, J. F., and J. E. Duffy. 2006. The central role of grazing in seagrass ecology, p. 463–501.*In* A. W. D. Larkum, R. J. Orth, and C. M. Duarte [eds.], Seagrasses: Biology, ecology and conservation. Netherlands: Springer.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology **80**: 1395–1404. doi:10.1890/0012-9658(1999)080%5B1395:PCCANA%5D2.0.CO%3B2

Vizzini, S., G. Sarà, R. H. Michener, and A. Mazzola. 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecol. **23**: 277–285. doi:10.1016/S1146-609X(02)01156-6

Waycott, M., C. M. Duarte, T. J. B. Carruthers, and others. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. **106**: 12377–12381. doi:10.1073/pnas.0905620106

Whalen, M. A., J. E. Duffy, and J. B. Grace. 2013. Temporal shifts in top-down vs. bottom-up control of epiphytic algae in a seagrass ecosystem. Ecology **94**: 510–520. doi:10.1890/12-0156.1

Williams, S. L., and M. H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology **74**: 904–918. doi:10.2307/1940815

Yan, J., T. van der Heide, B. Cui, J. Bai, T. Ysebaert, and J. Koppel. 2020. A healthy trophic structure underlies the resistance of pristine seagrass beds to nutrient enrichment. Limnol. Oceanogr. **65**: 2748–2756. doi:10.1002/lno.11545

Acknowledgments

This study was part of the *Zostera Experimental Network* (ZEN; www.zenscience.org) and was funded by the U.S. National Science Foundation (Grants OCE-1336206, OCE-1336741, and OCE-1336905). This is contribution 80 from the Smithsonian's MarineGEO and Tennenbaum Marine Observatories Network. Karine Gagnon was funded by the Maj and Tor Nessling Foundation (Grant 201900244), Camilla Gustafsson by the Maj and Tor Nessling Foundation (Grant 2008216) and Academy of Finland (Grant 295443), and Christoffer Boström by the Åbo Akademi University Foundation SR. We would like to thank Jonathan Lefcheck for advice on the biomass estimates. We would also like to thank the Archipelago Center Korpoström for providing excellent working facilities, and several anonymous reviewers for their constructive comments on this manuscript.

Conflict of interest

None declared.

Submitted 02 November 2020
Revised 23 February 2021
Accepted 30 April 2021

Associate editor: Anna Armitage